ON THE CONDITIONS OF ACTIVATION OF UNFERTILIZED STARFISH EGGS UNDER THE INFLUENCE OF HIGH TEMPERATURES AND FATTY ACID SOLUTIONS.[1]

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INTRODUCTORY.

In a former paper[2] I showed that brief exposure of the unfertilized eggs of *Asterias forbesii* to temperatures of 32° to 38° resulted in membrane-formation, cleavage and development. With normal eggs and the proper times of exposure almost every egg developed to a free-swimming larval stage; this treatment thus forms a highly effective parthenogenetic method. The time of exposure to the warm sea-water required to produce these effects is definite (within a certain slight range of variation) for any given temperature and decreases rapidly as the temperature rises. Thus, as regards the least exposure necessary for the formation of typical fertilization-membranes: "At 33° exposure must be prolonged to two minutes; at 34° the minimum lies somewhere between 30 and 60 seconds, at 35° between 15 and 30 seconds, at 37.5° between 5 and 15 seconds, and at 40° momentary exposure (5 seconds) produces membranes in practically all eggs."[3] The exposure required to induce development to larval stages was found to be considerably longer than for simple membrane-formation; at 35° from 70 to 90 seconds was required, at 36° from 50 to 60 seconds, at 37° from 30 to 35 seconds, and at 38° about 20 seconds. The responsiveness of the eggs to this form of treatment was found to depend on the stage of maturation; warming before the dissolution of the germinal vesicle had begun was ineffective and in fact inhibited maturation entirely; the most favorable period lay between the break-

[1] From the Marine Biological Laboratory, Woods Hole, and the Biological Laboratory, Clark University.

[2] *Journal of Experimental Zoology*, 1908, Vol. 5, p. 375.

[3] *Loc. cit.*, p. 384.

down of the germinal vesicle and the separation of the first polar body; after both polar bodies had separated development was imperfect and never proceeded far,—even membrane-formation then failed in many eggs.

Recent advances in the physiology of fertilization and artificial parthenogenesis have made it desirable to examine these effects of temperature in greater detail and to correlate them with the similar effects produced by other agents. During the past summer at Woods Hole I have accordingly re-investigated the changes in unfertilized starfish eggs following exposure for different periods to temperatures ranging from 28° to 36°, with especial reference to the differences in physiological effect resulting from differences in time of exposure to a given temperature (*e. g.*, 32°), and also with reference to the manner in which the time of exposure required to produce a given effect (*e. g.*, membrane-formation) varies at different temperatures. Determination of the temperature-coefficients of the processes underlying these effects is likely to afford indications of the nature of the fundamental changes concerned in the activation of the egg. Experiments on the effects of exposure to weak fatty acid solutions for different periods were also carried out; and on the action of high temperatures (32° to 34°) and fatty acid solutions on eggs which had previously been subjected to a membrane-forming treatment.

It is well known that the temperature-coefficients of a large number of physiological processes have been found similar to those of chemical reactions in general.[1] This result is to be regarded simply as an expression of the fact that the energy for such processes is usually chemical energy freed by oxidations or other reactions, whose rate accordingly determines that of the process in question. There are, however, many instances in which rise of temperature produces an entirely different kind of effect. Often a process exhibits a critical temperature below which it entirely fails to take place.[2] In such instances the

[1] For a summary account of researches in this field *cf.* C. D. Snyder: *American Journal of Physiology*, 1908, Vol. 22, p. 309.

[2] Examples of such processes are: inactivation of enzymes and toxines or destruction of microorganisms by heat; heat-coagulation of proteins, and dependent processes like injury or destruction of various cells by heat; onset of heat-rigor;

process may show a very rapid acceleration through a range of a few degrees above the critical temperature; it is then clear that the change of temperature acts in some other way than simply by accelerating an already existing chemical reaction. This is the class of cases to which belongs the influence of higher temperatures in initiating development in starfish eggs. Such departures from the usual temperature-coefficients of physiological processes indicate the entrance of other factors, the nature of which may be partly inferred from the character of the temperature-coefficient. Thus, to take the case of the starfish egg: in order to induce development of all eggs to a larval stage by exposure to a temperature of 31° it is necessary to keep them at this temperature for a period of about 15 minutes; at 36° an exposure of only one minute is necessary. The physiological process, whatever its nature, which renders the egg capable of proceeding with its development, thus takes place about fifteen times as rapidly at 36° as at 31°. This high temperature-coefficient indicates that a physical rather than a purely chemical change—possibly a change of the same nature as that determining the liquefaction of a warmed gel—is responsible for the altered behavior of the egg. The time-relations show that some definite and progressive process, the end-effect of which is to remove the conditions hindering further development, is taking place in the egg during the entire 15 minutes at 31°. Exposure for the full period of fifteen minutes is necessary to bring this process to its completion, *i. e.*, to a stage at which the egg is in a position, when returned to sea-water, to continue automatically its development to a larval stage. If the exposure is only 5 minutes there is also a definite change in the egg; a typical fertilization-membrane is formed and there may be some irregular change of form or possibly a few abnormal cleavages, but the egg never develops far and soon dies. In this case the process of activation is evidently incomplete, and only a few of the early steps in development are carried out. If the exposure is too long (20 to 25 minutes) the egg also fails to develop; the process initiated by the higher temperature thus

excitation of thermal sense-organs (*e.g.*, of frog's foot) and of certain vaso-motor and other temperature-regulatory mechanisms by heat; thermotactic responses.

gives rise to injurious conditions if it continues beyond a certain time. For each temperature, in fact (from 30° to 38°), there is a well-defined optimum duration of exposure which initiates favorable development in all normal eggs; also a briefer exposure which results in simple membrane-formation followed by breakdown; and a more prolonged exposure which renders the egg incapable of development. It is noteworthy that at each temperature the ratios of the durations required for these several effects are closely similar,—the optimum exposure being typically from two to three times that required for simple membrane-formation, and the maximum exposure (at which development to a larval stage just fails) about one and a half times the optimum.[1] This indicates that some single process, involving a critical change in the condition of the egg-protoplasm and having a characteristically high temperature-coefficient, underlies and conditions all of these effects. This process does not begin until a temperature of about 29° is reached, and proceeds slowly at that temperature, taking approximately 30 minutes to attain its completion. A rise of eight degrees accelerates it some hundred times. Such facts appear to narrow the range of possibilities very materially; they point clearly to some physical change,—of structure, colloidal aggregation-state, viscosity, etc. —rather than to one of a purely chemical kind, as constituting the critical process underlying the activation of the egg.

The experiments of the past summer have shown further that exposure to weak fatty acid solutions produces in the egg effects which are in all essential respects identical with those resulting from exposure to the above temperatures. Starfish eggs placed for one minute in sea-water containing $n/260$ butyric acid (2 c.c. $n/10$ butyric acid *plus* 50 c.c. sea-water) all form fertilization-membranes on return to normal sea-water; but if left without further treatment the eggs typically fail to cleave and soon break down without further development. Precisely the same effect is produced by brief exposure to warm sea-water, *e. g.*, three or four minutes at 32°. In either case it is necessary, in order to induce complete development of such eggs, to subject them to some second or supplementary treatment, such as

[1] *Cf.* below, page 279.

exposure to hypertonic or cyanide-containing sea-water. The starfish egg can, however, be made to develop completely without the necessity of any such after-treatment, simply by sufficiently prolonging the exposure to the membrane-forming agent. An exposure of 8 minutes to 32° is followed not only by membrane-formation, but by cleavage and development of all normal eggs to larval stages (*cf.* page 271). Similarly, exposure to *n*/260 butyric acid for a sufficient period—varying from 6 to 10 minutes —also causes all eggs to cleave and develop to larvæ (*cf.* p. 282). Over-exposure, if slight, is followed in both cases by a decrease in the proportion of favorably developing eggs; and if well-marked, by complete failure of development and early break-down. The only noteworthy difference that I have observed between the effects of the two agents is that the time-relations in the case of exposure to fatty acid have been somewhat more variable than in the case of exposure to a definite temperature such as 32°. Thus in some experiments eggs have exhibited a considerable proportion of favorable developments after only one minute's exposure to weak fatty acid solutions.[1] In such cases however the concentration of acid was somewhat higher (3 c.c. *n*/10 fatty acid *plus* 50 c.c. sea-water) than in the experiments described above. In last summer's experiments (in which the fatty acid was always used in *n*/260 concentration) the curves relating time of exposure to the proportion of eggs forming larvæ were virtually identical in form with the two agents,—a fact showing that the essential effects produced by both types of treatment are the same.

The fact that a properly timed single exposure to warm sea-water or fatty acid solution causes complete development suggests that the necessity for a supplementary after-treatment (*e. g.*, with hypertonic sea-water), in the case of eggs in which fertilization-membranes have been formed by brief preliminary exposure to a cytolytic agent, depends simply on the incompleteness of the change induced in such eggs by the membrane-forming treatment. The fact that by sufficiently prolonging this treat-

[1] See the experiments described in my recent paper in the *Journal of Experimental Zoology*, 1913, Vol. 15, pp. 41, 42. Starfish eggs exposed for 1 minute to a mixture of 3 c.c. *n*/10 acetic or butyric acid plus 50 c.c. sea-water (*n*/176 acid) gave in several cases 20–30 per cent. of larvæ and in one case 70–80%.

ment one can induce complete development in all eggs indicates clearly that the after-treatment produces in the egg effects which are physiologically of the same kind as those resulting from the membrane-forming treatment, and not qualitatively different as has usually been supposed. If this is so, we must conclude that hypertonic sea-water is favorable not because it exerts a "corrective" action different from that of the membrane-forming agent, but simply because it enables the process started by the first treatment and arrested at an unfinished stage to proceed to its completion. On this view the effects of the two successive treatments are simply additive. Apparently under the influence of the higher temperature or the fatty acid a certain definite process, which we may call the activation-process, is started in the egg. This process, if it proceeds to a certain definite stage, puts the egg in a condition to continue automatically its development to the formation of larvæ; but if the process is arrested too soon (by the return to sea-water), the egg is able to carry out only a few of the early steps of development, including membrane-formation and perhaps a few cleavages. The after-treatment merely causes the resumption of the process and carries it to its completion. The unitary character of the activation-process is further indicated by the fact that the temperature-coefficients for simple membrane-formation and for the complete initiation of development are the same, as will be shown below. If this conclusion is correct, it should be a matter of indifference whether the exposure for the required period to the high temperature or the fatty acid solution is continuous or discontinuous. It ought to be possible to form fertilization-membranes by brief exposure to warm sea-water or fatty acid followed by a return to normal sea-water, and then later to complete the activation-process by a second exposure to either agent for an appropriate time. This is in fact the case; all of the four combinations have been tried: brief treatment with warm sea-water followed by after-treatment for several minutes with either warm sea-water or butyric acid solution; and membrane-formation by butyric acid followed by warming or a second treatment with acid. All four methods give the same result, namely the development of a high proportion of eggs to

larval stages. The effect of such second treatment is in fact indistinguishable from that of exposure to hypertonic sea-water or cyanide.

The problem of the nature of the effect produced on the egg by hypertonic sea-water, or the other corrective agent employed to supplement the membrane forming treatment, thus appears in a simpler light. In the starfish egg, after membranes have been formed as above, an exposure to (*e. g.*) 32° or to weak butyric acid solution for several minutes constitutes a highly favorable form of after-treatment, producing the same effect on development as hypertonic sea-water or cyanide.[1] This makes it appear doubtful that two qualitatively distinct processes are concerned in the activation of other eggs like the sea-urchin egg, where some form of after-treatment, different from the membrane-forming treatment, has hitherto proved necessary in order to induce development in a high proportion of eggs. The conditions are unlikely to be fundamentally different in the two animals. In the starfish egg the "corrective" effect resulting from after-treatment by heat has the same high temperature-coefficient as the initial change underlying simple membrane-formation by heat.[2] This could hardly be the case if the two processes were qualitatively dissimilar; it indicates clearly that the same fundamental change in the egg-protoplasm furnishes the conditions for both the membrane-forming process and the "corrective" process. I have found that in the *Arbacia* egg temporary warming (1 to 6 minutes at 32°, 34° and 35°) does not cause development (except in very few cases) even if followed by hypertonic sea-water;[3] and there is no evidence that prolonged treatment with weak fatty acid solutions will cause complete development in this egg.[4] The only highly and invariably

[1] *Cf.* the experiments summarized in Tables XIII to XVII below.

[2] Compare the experiments of Tables XIV and XV below.

[3] Unpublished experiments performed last summer. An occasional egg may form a larva under this treatment, but the great majority remain unaltered.

[4] In the case of *Strongylocentrotus purpuratus* Loeb found that eggs exposed to butyric acid solutions of the concentrations $n/250$, $n/166$, and $n/125$ for more than 2 minutes failed to form membranes ("Artificial Parthenogenesis and Fertilization," p. 141). Herbst found that eggs of *Sphærechinus* treated for 2, 5, and 8 minutes with a mixture of 50 c.c. sea-water *plus* 3 c.c. $n/10$ acetic acid gave only occasional larvæ (*Roux's Archiv*, 1906, Vol. 22, p. 473). Apparently no systematic experi-

effective after-treatment hitherto discovered for the sea-urchin egg is hypertonic sea-water.[1] It would thus appear that the conditions in this egg differ considerably from those in the starfish; but the fact that a simple exposure to hypertonic sea-water, if sufficiently prolonged, has the same effect in inducing development as a briefer exposure to the same agent combined with membrane-formation by fatty acid, seems to indicate that the conditions are fundamentally similar in both types of egg, and that a unitary process underlies activation in both cases. The remarkable effectiveness of hypertonic sea-water with the sea-urchin egg would seem to be due to certain special largely incidental pecularities; temporary abstraction of water appears for some reason to render this egg more resistant to the dissolution that otherwise results from the membrane-forming treatment.[2] In other eggs, however, like those of the starfish or *Nereis*, hypertonic sea-water shows no special advantages over a number of other forms of after-treatment. The fact that a double form of treatment has hitherto proved especially effective with the sea-urchin egg is thus not inconsistent with the view that the activation-process is essentially unitary in character in all eggs.

EXPERIMENTAL. EFFECTS OF SIMPLE EXPOSURE TO WARM SEA-WATER.

In these experiments the procedure was similar to that described in my earlier paper.[3] Sea-water at a temperature slightly above that chosen for the experiment was added rapidly to the small beaker containing the eggs (with a thermometer) until the

ments of this kind have yet been performed with *Arbacia*. At Naples, using *Arbacia pustulata*, Lyon was able to cause development to larvæ in *ca.* 10 per cent. of eggs by exposure to sea-water acidulated with HCl, but he did not try fatty acids (*Amer. Journ. Physiol.*, 1903, Vol. 9, p. 310).

[1] Cyanide is only slightly effective with *Arbacia punctulata* (*cf.* my experiments described in *Journal of Morphology*, 1911, Vol. 22, page 703); it is more so with *Strongylocentrotus*, according to Loeb's results (*cf.* "Artificial Parthenogenesis and Fertilization," p. 80), but even here it is less uniformly favorable than hypertonic sea-water.

[2] *Cf.* the experiments of Loeb (*loc. cit.*, Chapter XI; also *Archiv für Entwicklungsmechanik*, 1914, Vol. 38, p. 409). It is probable that hypertonic sea-water has another and more distinctive mode of action (see below, p. 300).

[3] *Journal of Experimental Zoology*, 1908, Vol. 5, p. 379.

required temperature was reached; this temperature was then kept constant during the period of the experiment by immersing the beaker in a water-bath at the same temperature. At intervals eggs were transferred to sea-water at room-temperature contained in finger-bowls. The exposure to the warm sea-water always took place during the interval between the complete disappearance of the germinal vesicle and the formation of the first polar body.

Exposure to 28°, even if prolonged to 45 minutes, proved almost entirely ineffective in forming membranes in starfish eggs. With exposures of 30 minutes or more an occasional egg may form a membrane, but the great majority always remain unaltered.

At 29° membranes appear in a considerable proportion of eggs after exposures of 12 to 15 minutes. With longer exposures (25 to 30 minutes) a majority in some cases (not always) may form membranes, and a considerable number may develop to larval stages. Table I. summarizes the results of two series of experiments in which eggs were exposed to 29° for periods ranging from 2 to 40 minutes. In both lots of eggs the great majority underwent normal maturation, and a large proportion developed normally to larvæ after sperm-fertilization.

TABLE I.

29°.

Duration of Exposure in Minutes.	Approximate Proportion of Eggs Forming Fertilization-membranes and Larvæ.			
	Series of June 10.		Series of June 11.	
	Membranes.	Larvæ.	Membranes.	Larvæ.
2 to 10 m.	0	0	0	0
12 m.	*ca.* 2–3%	0	*ca.* 1%	0
14 m.	10– 15%	0	*ca.* 3–4%	0
17 m.	25– 30%	0	*ca.* 10%	0
20 m.	*ca.* 50%	<1%	*ca.* 20%	0
25 m.	70– 80%	*ca.* 2–3%	5–10%	*ca.* 1%
30 m.	30–40%	15–20%	few	*ca.* 5%
40 m.	*ca.* 1–2%	*ca.* 1–2%	0	0

The two series show some minor differences, but in both the number of eggs forming larvæ is small, and a certain proportion fail to form membranes even with the optimal exposures. This temperature is near the lower limit below which the eggs show no response to this form of treatment.

At 30° the proportion of eggs forming membranes and develop- to larval stages is higher than at 29°, although considerable variability is still shown. Five series of experiments were performed at this temperature. Table II. summarizes the results of four of these.[1] Each lot of eggs was favorable, maturation and development to larvæ after sperm-fertilization taking place in nearly all. Table II. gives the approximate proportion of eggs forming membranes and developing to blastulæ after exposure for the periods given in the first column.

TABLE II.

30°.

Duration of Exposure in Minutes.	Proportion of Eggs Forming Fertilization-membranes and Larvæ.							
	June 7.		June 8.		June 12.		June 13.	
	Membranes.	Larvæ.	Membranes.	Larvæ.	Membranes.	Larvæ.	Membranes.	Larvæ.
Up to 3 m.	0	0	0	0	0	0		
4 m.	ca. 5%	0	0	0	0	0		
5 m.	15– 20%	0	0	0	0	0	<1%	0
6 m.	ca. 20%	0	0	0				
7 m.	ca. 30%	0			ca. 5%	0	<1%	0
8 m.	ca. 80%	0	ca. 1%	0			5–10%	0
9–10 m.	ca. 100%	ca. 2–3 %	20–25%	0	40–50%	0	10–15%	0
12 m.			50–60%	ca. 1%	70–80%	0	20–25%	<1%
14–15 m.	ca. 100%	ca. 5%	80–90%	2– 3%	>90%	10–15%		
17–18 m.			ca. 90%	5–10%			30–40%	ca. 5%
20–21 m.			<50%	5–10%			ca. 40%	ca. 30%
24 m.							30–40%	30–40%
28 m.							ca. 40%	ca. 40%
30 m.			ca. 10%	<5%				
34 m.							ca. 15–20%	<1%

It will be noted that in four out of the five series at 30° an exposure of 8 to 10 minutes was required to cause membrane-formation in 10 per cent. or more of the eggs; in the fifth series (June 7) 5 minutes was sufficient. The proportion of eggs developing to larvæ was comparatively low in all series; the optimum exposure lay between 24 and 28 minutes in the only series (June 13) in which the proportion of larvæ was considerable. With longer exposures membranes become fewer and there is a rapid decline in the proportion of eggs forming larvæ.

[1] In the remaining series the longest exposure was 10 minutes, at which about two thirds of the eggs formed membranes and a small number developed to larvæ.

At 31° the conditions become more favorable and with the proper times of exposure practically all mature eggs form fertilization-membranes, and in favorable cases the great majority develop to larvæ. Four series of experiments were performed at this temperature; in one of these (August 28) only about half the eggs underwent maturation, and with 15 minutes' exposure (approximately the optimum) only 10 to 15 per cent. of all eggs formed larvæ. In the other three series the eggs were normal. The proportions of eggs forming membranes and larvæ in these series with the different times of exposure are given in Table III.

TABLE III.

31°.

Duration of Exposures in Minutes.	Proportion of Eggs Forming Fertilization-membranes and Larvæ.					
	June 8.		June 12.		June 13.	
	Membranes.	Larvæ.	Membranes.	Larvæ.	Membranes.	Larvæ.
1–2 m.	0	0	0			
2½ m.			0		0	0
3 m.	0	0	*ca.* 10–15%		*ca.* 5%	0
3½ m.			30– 40%		*ca.* 50%	0
4 m.	Few (<1%)	0	70– 80%		60– 70%	0
5 m.	10–15%	0	*ca.* 90%		>95%	0
6 m.	*ca.* 20%	0	$\geqq$90%		*ca.* 100%	0
8 m.	80–90%	*ca.* 2–3%	> 90%	*ca.* 1%	*ca.* 100%	*ca.* 1%
10 m.	*ca.* 90%	15–20%	*ca.* 100%	20–30%	*ca.* 100%	*ca.* 20%
12 m.	*ca.* 90%	40–50%			*ca.* 100%	*ca.* 60%
14–15 m.	70–80%	40–50%			*ca.* 90%	80–90%
17–18 m.	*ca.* 50%	*ca.* 40%			*ca.* 75–80%	50–60%
20–21 m.	15–20%	*ca.* 5%			40–50%	*ca.* 10–15%
25–30 m.					*ca.* 20%	0

At this temperature an exposure of 3 to 4 minutes is required to cause membrane-formation in 10 per cent. or more of the eggs; exposure must be prolonged to *ca.* 8 minutes before any eggs form larvæ; 14 to 15 minutes is the approximate optimum. In the series of June 12 this optimum was not reached.

At 32° a larger number of experiments were performed than at any other temperature, and their results show a decidedly greater uniformity than at lower temperatures. With the optimal times of exposure (from 7 to 8 minutes) the proportion of larvæ yielded by normal eggs is always high,—usually over 90 per cent. This is illustrated by Table IV., which summarizes the results of six successive series performed during June at a

time when starfish eggs were unusually abundant and favorable. On account of the relative completeness of my observations at this temperature, the general results of these experiments will be described in some detail.

The exposure required for membrane-formation is about half that at 31°. An exposure of 3 minutes typically forms membranes in all normal eggs, and one of 2 minutes is usually sufficient to produce this effect in a minority and sometimes in a majority of eggs. From 3 minutes on the conditions remain normal for membrane-formation until the exposure is prolonged to 12 or 15 minutes, after which in a certain proportion of eggs membranes tend to separate imperfectly or even fail to form. In most series exposures longer than 15 minutes were not used, since eggs so treated never form larvæ; in one series, however, eggs were exposed for 27 minutes, at which exposure nearly half failed to form membranes. This decline in membrane-formation when exposures are prolonged beyond a certain maximum is general for all temperatures (*cf.* also the series at 30°, 31° and 33°); the fact is interesting since it indicates that the process is not a direct effect of the high temperature but constitutes an active response —probably in the nature of a secretion—on the part of the egg.

The optimum exposure for inducing complete development at 32° varies between 6 and 8 minutes, and with this exposure the great majority of normal eggs cleave and develop to larval stages. Many of the gastrulæ and Bipinnariæ thus obtained are apparently quite normal and swim freely at the surface of the water. The rate of development is, however, always slower than that of sperm-fertilized eggs; relative slowness of development seems in fact to be a constant peculiarity of parthenogenetically activated eggs.[1] Exposures well above the optimum are followed by imperfect or delayed cleavage and failure to develop beyond early stages.

Table IV. gives a summary of the results of the six successive series referred to above. The approximate proportions of eggs forming free-swimming larvæ are given; the conditions of membrane-formation have already been sufficiently described.

[1] This has been my uniform experience since I began studies of this kind, and apparently the experience is general. This suggests strongly that the spermatozoon contributes to the egg material which is utilized in normal development.

TABLE IV.

32°.

Time of Exposures.	Proportion of Eggs Forming Free-swimming Larvæ.					
	June 12.	June 13.	June 18.	June 24.	June 25.	June 26.
1–3 m.	0	0	0	0	0	0
4 m.	*ca.* 1%	<1%	*ca.* 4–5%	2– 3%	*ca.* 5%	0
5 m.	*ca.* 3– 4%	2– 3%	15–20%	25–35%	*ca.* 50%	10–15%
6 m.	*ca.* 35–40%	20–30%	55–60%	60–70%	80–90%	25–35%
7 m.		70–80%		≧90%	*ca.* 60%	50–60%
8 m.	>90%	*ca.* 95%	*ca.* 95%	≧90%	25–35%	80–90%
10 m.	85–90%	50–55%	75–85%	50–60%	< 5%	80–90%
12 m.		15–20%	25–35%		0	*ca.* 20%
15 m.		0	<1%			
18, 22, and 27 m.		0	0			

These results may safely be regarded as typical. Six other similar series were carried out at this temperature. In two of these the eggs were unfavorable or the treatment was applied too late. In the four others—two in early June and two in late August—the results were similar to the above, although fewer eggs formed larvæ; the optimum exposures ranged from 6 to 8 minutes, with respectively 20, 20, 40 and 50 per cent. of mature eggs forming larvæ. It will be noted that the optimum exposure is approximately 8 minutes in five out of the six series in Table IV. Different lots of eggs vary somewhat in the duration of this optimum; thus in the series of June 25 half of the eggs formed larvæ with only five minutes' exposure and the optimum was 6 minutes, and on June 26 the eggs showed almost equally good development with the 8- and the 10-minute exposures. In the majority of series, however, there was a well-defined optimum at 7 or 8 minutes.

The physiological effects following exposure to 32° vary in a constant and highly characteristic manner with the duration of the exposure. Eggs exposed for a period insufficient to induce membrane-formation show no apparent change on return to sea-water and later break down without development. Such eggs, however, can be shown to have undergone some internal change similar in kind to that following longer exposures; thus if later they are again exposed to 32° they are found to require, in order to induce favorable development, a shorter exposure

than previously untreated eggs (*cf.* below, p. 288). Exposure for 3 to 4 minutes induces typical membrane-formation in all eggs, followed, however, not by cleavage and further development but by irregular changes of form, fragmentation, and eventual breakdown. With somewhat longer exposures (4 to 5 minutes) membrane-formation is followed by symmetrical cleavage in a certain proportion of eggs; and the proportion of such cleavages, and also their approximation to the normal in rate and character, show a progressive increase with increasing length of exposure up to the optimum of about 8 minutes. With still longer exposures the response again becomes unfavorable, and eventually the eggs entirely fail to develop and even to form membranes. We have here an apparent reversal of the rule enunciated by Loeb with reference to the action of membrane-forming agents on the sea-urchin egg. "A relatively brief exposure to a cytolytic agent leads only to membrane-formation, while a longer exposure causes cytolysis."[1] In the starfish egg a relatively brief exposure to warm sea-water (one just sufficient for membrane-formation) is followed by an early cytolysis, while a longer exposure results not only in membrane-formation but in an approximately normal development; still longer exposures again cause cytolysis without development. This rule applies to the action of cytolytic substances like fatty acid, as well as to high temperatures (*cf.* below, p. 282).

To illustrate the effects of exposures of different duration on cleavage the following record is given (Table V.) describing the condition of the eggs about four hours after exposure to 32° for the times given.

It will be noted that with brief exposures (3 to 4 minutes) membrane-formation is typical, but the eggs are unable to cleave normally and undergo irregular change of form followed by breakdown. As the time of exposure increases, an increasing proportion of eggs cleave, until the optimum (6 to 7 minutes) is reached at which cleavage approaches the normal in rate and character, and the great majority develop to larval stages. Over-exposure (10 minutes) is again followed by failure of cleavage and development. Similar observations were made

[1] "Artificial Parthenogenesis and Fertilization," 1913, p. 8.

in experiments at other temperatures; in all cases the exposure which induced the largest proportion of regular cleavages was found to correspond with that at which the largest proportion of eggs formed larvæ. In the series of Table V. the optimum, 6 minutes, is somewhat shorter than usual; on June 24 the optimum of cleavage was found at 7 minutes, and on June 26 at 8 minutes, with 10 minutes somewhat less favorable.

TABLE V.

JUNE 25. 32°.

Time of Exposure.	Condition of Eggs 4 Hours after Exposure, and Proportion of Eggs forming Larvæ.
1. 2 m.	Great majority are unchanged; a few have membranes. No larvæ.
2. 3 m.	Most eggs have typical membranes and are irregular or amœboid in form; a few show irregular cleavages. No larvæ.
3. 4 m.	Almost all eggs have membranes and exhibit irregular forms; a few have cleaved symmetrically. *Ca.* 5 per cent. form larvae.
4. 5 m.	Marked improvement over Experiment 3: most eggs have cleaved, and many are in regular 4- and 8-cell stages. *Ca.* 50 per cent. form larvæ.
5. 6 m.	Almost all eggs are cleaved; cleavages are more regular and advanced than in Exp. 4; 16-cell stages are frequent. 80–90 per cent. form larvæ.
6. 7 m.	The proportion of regular cleavages is also high, but rather less than in Exp. 5. *Ca.* 60 per cent. form larvæ.
7. 8 m.	Cleavages are fewer and less advanced than in Exp. 6. *Ca.* 25–30 per cent. form larvæ.
8. 10 m.	Great majority are uncleaved; many are irregular in form or fragmented. Larvæ are few: $<$ 5 per cent.
9. 12 m.	Almost none have cleaved. The eggs are largely irregular or with small surface-vesicles detached.

While an exposure just long enough for membrane-formation is insufficient by itself to induce normal cleavage and development, it is possible, after forming membranes in this way, to make the eggs cleave and develop to larval stages by subjecting them to a second treatment with warm sea-water, or by after-treatment with fatty acid (n/260 butyric acid in sea-water), hypertonic sea-water, or cyanide (n/1000 KCN in sea-water). These effects will later be described in detail (*cf.* Tables XIII., XVII.).

It is interesting to note that the effects produced by weak fatty acid solutions (*n*/260 butyric acid) on unfertilized starfish eggs also vary with the time of exposure in a manner closely similar to that just described. Brief exposure causes membrane-formation followed by irregular change of form and breakdown without development, while longer exposure induces not only membrane-formation but cleavage and development to larval stages; still longer exposure is again unfavorable. Eggs in which membranes have been formed by the minimal exposure to fatty acid may be made to develop by the above forms of after-treatment. The effects of the two agents, warm sea-water and weak butyric acid solution, seem in fact to be identical in every essential particular, and the one may be substituted for the other without altering the effect on the egg (*cf.* Tables XIV. to XVII.). Experiments showing this parallelism will be described in detail later. There is in fact every indication that the underlying physiological process which enables the egg to continue normal development is of the same nature as that which induces simple membrane-formation, the only difference being that the duration of the process must be considerably longer in the second case than in the first. The temperature-coefficients of both effects indicate the same, as will appear below (*cf.* Table X.). The possible nature of this process will be discussed in the concluding section of this paper.

Treatment with sea-water at 33° gives similar results to those above described, except that the times required to produce a given physiological effect are only a little more than half as long as at 32°. An exposure of from one to one and a half minutes is needed to call forth membrane-formation in the majority of eggs. Four series of experiments with normal eggs were performed at this temperature, and in every series the great majority of eggs formed larvæ with the optimal times of exposure. In these series the earlier transfers from the warm sea-water to normal temperature were made at half-minute intervals. The results are summarized in Table VI.

In all of these series the proportion of favorably developing eggs is high with the optimum exposures of 4½ to 5½ minutes. The series of June 15 is unusual in that nearly all of the eggs

form larvæ with exposures varying in length from 4 to 6 minutes. The optima seem to be less sharply defined when the eggs are in the best of condition, probably because then the power of regulatory adjustment to environmental variations is at its maximum, and slight deviations from the optima are automatically corrected.

TABLE VI.

33°.

Time of Exposure.	Proportion of Eggs Forming Larvæ (Blastulæ and Gastrulæ).			
	June 9.	June 10.	June 15.	June 17.
1–1½ m.	0	0	0	0
2 m.	0	Very few (<0.1%)	0	0
2½ m.	Few (<1%)	*ca.* 1%	< 1%	
3 m.	10–15%	*ca.* 10%	*ca.* 5%	25–30%
3½ m.	*ca.* 50%	50–60%	20–30%	
4 m.	80–90%	80–90%	*ca.* 90%	55–65%
4½ m.	60–70%	⋛90%	*ca.* 90%	
5 m.	40–50%	90–95%	*ca.* 95%	90–95%
5½ m.			90–95%	
6 m.	20–25%	>90%	*ca.* 90%	*ca.* 95%
7 m.	*ca.* 2–3%		75–85%	
8 m.		70–80%	10–15%	70–80%
10 m.		15–20%	0	30–40%
12 m.			0	
15, 18, 21 and 25 m.			0	

At 34° the majority of eggs form membranes with one minute's exposure, and 30 seconds is sufficient for a minority. A few eggs form larvæ after 2 minutes' exposure; the optimum is 3 to 4 minutes; longer exposure is injurious. Table VII. summarizes

TABLE VII.

34°.

Time of Exposure.	Proportion of Eggs Forming Larvæ.			
	June 10.	June 15.	August 26.	August 27.
30, 1 m.	0	0		
1½ m.	*ca.* 1%	0		
2 m.	25–35%	0	0	*ca.* 1%
2½ m.	50–60%	20–30%	*ca.* 1%	
3 m.	65–75%	*ca.* 90%	5–10%	25–30%
3½ m.	70–80%	*ca.* 90%	10–15%	
4 m.	50–60%	*ca.* 90%	15–25%	35–40%
4½ m.		*ca.* 90%	20–30%	
5 m.	5–10%	*ca.* 10%	20–30%	*ca.* 5%
5½ m.		*ca.* 1%		
6 m.	<1%	0	10–15%	0
7, 8, 10 and 12 m.	0	0	0	

the results of four series of experiments at this temperature. The August eggs were less favorable than the June eggs.

Two similar series at 35° and one at 36° were carried out in June at a time when starfish eggs were unusually favorable. The results were similar to those at 33° and 34° except that the physiologically equivalent exposures were shorter. At 35° an exposure of 30 seconds induces membrane-formation in many but not in all eggs, and one of 45 seconds in practically all. At 36° 15 seconds is sufficient to form membranes in about half the eggs and 30 seconds in all. Longer exposures eventually interfere with membrane-formation; thus after 6 to 8 minutes at 36° membranes failed to form or were imperfect in 40 to 50 per cent. of eggs. Table VIII. gives the proportions of eggs forming swimming larvæ in these experiments. The transfers from warm to normal sea-water were made at first at intervals of fifteen seconds.

Table VIII.

35° and 36°.

Time of Exposure.	Proportion of Eggs Forming Larvæ.		
	June 15 (35°).	June 16 (35°).	June 17 (36°).
15″, 30″	0	0	0
45″	0	0	20–30%
1 m.	*ca.* 1%	0	85–90%
1 m. 15″	*ca.* 5%	*ca.* 4–5%	$\geqq$95%
1 m. 30″	80–90%	35–45%	70–80%
1 m. 45″	>95%	70–80%	*ca.* 10%
2 m.	*ca.* 90%	$\geqq$90%	none free
$2\frac{1}{2}$ m.	*ca.* 50%	40–50%	0
3 m.	*ca.* 5%	5–10%	0
$3\frac{1}{2}$ m.	0	0	0
4 m.–10 m.	0	0	0

The rapid decrease in the optimum exposures as the temperature rises is to be noted; the optima are respectively $1\frac{1}{2}$ to 2 minutes at 35°, and 1 to $1\frac{1}{4}$ minutes at 36°.

Variation with Temperature in the Rate of the Process Underlying Activation by Heat.

The foregoing results show that the process, whatever its nature, which is initiated in the mature unfertilized starfish egg by temperatures of 30° to 36°, and which brings the egg into a condition to form membranes cleave, and develop, proceeds

twenty or twenty-five times as rapidly at 35° as at 30°. Thus at 30° the minimum exposure for membrane-formation is about 10 minutes, and at 35° 30 seconds or less; similarly at 30° the minimum exposure needed to induce even a few eggs to form larvæ is about 20 minutes, at 35° it is about 1 minute; at 30° the optimum exposure is *ca.* 30 minutes, and at 35° *ca.* 1½ minutes. For each temperature it is possible to assign a definite length of exposure which produces a definite effect on the egg. The manner in which these times of exposure vary at different temperatures may be seen by reference to Table IX. Here are

TABLE IX.

Temperature and Series.	Minimum for Membrane-formation.	Minimum for Larvæ.	Optimum for Larvæ.	Maximum for Larvæ.
29° (June 10)	14 m.	25 m.		
30° (June 13)	8–10 m.	12–18 m.	24–28 m.	
31° (June 8)	5 m.	8 m.	12–14 m.	20 m.
(June 12)	3 m.	8 m.		
(June 13)	3½ m.	8 m.	14–15 m.	21 m. (<25 m.)
32° (June 12)	2½ m.	4 m.	8 m.	
(June 13)	2 m.	4–5 m.	8 m.	12 m. (<15 m.)
(June 18)	2 m.	4 m.	8 m.	12 m. (<15 m.)
(June 24)	2–3 m.	4 m.	7–8 m.	? (>10 m.)
(June 25)	2 m.	4 m.	6 m.	10 m. (<12 m.)
(June 26)	3 m.	5 m.	8–10 m.	12 m.
33° (June 9)	2 m.	3 m.	4 m.	7 m.
(June 10)	1 m.	2½ m.	4½–6 m.	10 m.
(June 15)	1 m.	3 m.	4–6 m.	8 m. (<12 m.)
(June 17)	1 m.	3 m.	5–6 m.	10 m.+
34° (June 10)	30 sec.	1½ m.	3–3½ m.	5 m. (<6 m.)
(June 15)	1 m.	2½ m.	3–4 m.	5½ m. (<6 m.)
35° (June 15)	30 sec.	1 m.	1 m. 30″–1 m. 45″	3 m. (<3½ m.)
(June 16)	30 sec.	1 m. 15″	*ca.* 2 m .	3 m. (<3½ m.)
36° (June 17)	15 sec.	45″(>30″)	1 m.–1 m. 15″	*ca.* 2 m. (<2½ m.)

tabulated the observations made in all of those series of experiments in which a large proportion of eggs formed larvæ,—in which, therefore, the conditions may be regarded as essentially normal. In the series at 29° few eggs formed larvæ; at 30° only one series out of five gave a considerable proportion of larvæ (*ca.* 40 per cent.) with *ca.* 30 minutes' exposure; in all of the other series in the table, except one at 31°, the great majority of eggs—usually over 90 per cent.—formed larvæ with the optimal exposures. In the first column is given the least time of exposure required for membrane-formation in a significant pro-

portion of eggs—10 per cent. or more; in the second column the least exposure at which any eggs (> 1 per cent.) formed larvæ; in the third the optimal time of exposure; and in the fourth the longest observed exposure at which any eggs (> 1 per cent.) formed larvæ.

If the several observed durations at each temperature are averaged, the following results are obtained (Table X.); the values are given in approximate terms rather than strict arithmetical averages, to emphasize the fact that the precise durations vary to a certain degree, even in normal eggs. There is, however, for each temperature a well-defined modal duration of exposure for producing a definite physiological effect such as membrane-formation or complete activation.

TABLE X.

APPROXIMATE TIMES OF EXPOSURE REQUIRED TO PRODUCE THE FOLLOWING EFFECTS AT DIFFERENT TEMPERATURES.

Temperature.	Formation of Membranes.	Minimum for Larvæ.	Optimum for Larvæ.	Maximum for Larvæ.
29°	*ca.* 12–14 m.	20–25 m.	30–40 m.	
30°	8–10 m.	*ca.* 18 m.	*ca.* 28 m.	? >30 m.
31°	*ca.* 4 m.	*ca.* 8 m.	*ca.* 15 m.	21–25 m.
32°	*ca.* 2 m.	4– 5 m.	7– 8 m.	10–12 m.
33°	*ca.* 1 m.	2½– 3 m.	4½–5½ m.	8–10 m.
34°	30″ to 1 m.	*ca.* 2 m.	3–3½ m.	*ca.* 5 m.
35°	*ca.* 30″	1–1¼ m.	1½–2½ m.	*ca.* 3 m.
36°	*ca.* 15″	30″–45″	1–1¼ m.	*ca.* 2 m.
37°			30–35 sec.	
38°			*ca.* 20 sec.	

It will be noted (1) that for each temperature there is a minimum effective exposure which induces membrane-formation followed by failure to develop and early breakdown; and (2) that an exposure of approximately twice the minimum for membrane-formation is required to enable even a few eggs to develop to larval stages, and an exposure of three or four times this minimum to enable development to proceed normally in all eggs; and (3) that if the exposure is prolonged to about one and a half times this optimum the eggs are again incapacitated from further development. The fact that the ratios of the durations required to produce these several effects are approximately the same at any one temperature indicates that a single process

of a definite kind forms the determining condition of all. This process is peculiar in undergoing marked acceleration by slight rise of temperature; it is also clear, from the fact that an effective exposure must last for a certain minimal time at any temperature, that the process must proceed to a definite stage before the egg is rendered capable of continuing its development to advanced stages; if the process is arrested before its completion, only the earlier developmental changes can be carried out (membrane-formation, early cleavage or change of form); if, on the other hand, it is allowed to proceed too far, injurious conditions arise which eventually prevent all development; a sufficiently prolonged exposure to high temperature renders the egg incapable even of membrane-formation.

In endeavoring to form some consistent conception of the nature of this process the following facts have to be considered. It exhibits a high temperature-coefficient: from fifteen to twenty times the duration of exposure is required to induce membrane-formation at 30° as at 35°; the ratios between 29° and 34° and between 31° and 36° are the same. At each temperature the proportionate durations of the minimum, optimum, and maximum exposures for forming larvæ are approximately the same. In other words, the critical change underlying simple membrane-formation is affected by temperature in the same way as that underlying complete activation of development: *i. e.*, the proportionate increase in velocity by rise of temperature is the same in both cases, a fact which can only indicate that one fundamental process—and not two—is concerned in producing both effects. If we assume that the above proportionate increase in velocity prevails through a rise of 10°, a Q_{10} value of from 225 to 400 is indicated, as against the 2 to 3 characteristic of chemical reactions in homogeneous media.[1] Activation by heat thus

[1] The temperature-coefficients of the rate of cytolysis of sea-urchin eggs and of the duration of life of sea-urchin larvæ and of Tubularia stems at temperature of 25° to 40° show similarly high values. In these cases the direct effect produced on the cell by the high temperature is probably of the same kind as that underlying the above activation-effect; this appears to be a change in the colloids of the plasma-membranes, leading to an increase of permeability. (See below, p. 296.) Such a change if not reversed within a certain time results in cytolysis. In the unfertilized starfish egg *temporary* increase of permeability involves activation. For data on the temperature-coefficients of cytolysis and heat-death, *cf.* J. Loeb,

depends on some critical change in the egg which does not begin until a temperature of about 29° is reached, but which undergoes very rapid acceleration with further rise of temperature. The liquefaction of gels by heat seems to be the only relevant process which shows these characteristics. The change in viscosity preceding the gelation of a gelatine sol undergoes very rapid acceleration with lowering of temperature, within a few degrees of the temperature of gelation. The inverse process, melting of gels, has a similarly high temperature-coefficient (*cf.* below, p. 295). In general the facts suggest that the direct effect of the high temperature is to cause a change in the colloidal system of the egg, of such a kind as to render possible a chemical interaction between substances which in the normal condition of the resting egg are kept apart. This restraining condition may be some physical barrier like a membrane, impermeable to the diffusion of the substances concerned, or it may be a certain state of electrical polarization of the general cell-surface, as suggested below (p. 299). It is also important to note that the activation-process may be arrested by a return of the eggs to sea-water at ordinary temperatures, and renewed after an interval without interfering with its effect. A reversibility of the physico-chemical change forming its basis is thus indicated. It should further be noted that cytolytic agents like butyric acid not only have the same general physiological effect as brief warming, but that the relations between time of exposure and physiological effect produced are the same in both cases. Some process which is affected similarly by these two dissimilar agents is thus to be sought. In the following section the results of experiments with weak butyric acid solution are described in greater detail.

Effects of Exposure to Butyric Acid Solution for Different Periods.

As already stated, treatment of starfish eggs during the early maturation period with weak solutions of butyric acid in sea-water ($n/260$) produces the same effects as temporary warming,

Archiv f. d. ges. Physiologie, 1908, Vol. 124, p. 411; A. R. Moore: *Quarterly Journal of Experimental Physiology*, 1910, Vol. 3, p. 257; *Arch. f. Entwicklungsmech.*, 1910, Vol. 29, pp. 146, 287.

and the time-relations of the exposures necessary for these effects are closely similar with both methods. Table XI. summarizes the results of five series of experiments with separate lots of eggs. The eggs were exposed at normal temperatures (20° to 22°) to an *n*/260 solution of butyric acid in sea-water (50 c.c. sea-water *plus* 2 c.c. *n*/10 butyric acid), and portions were transferred to normal sea-water at the intervals named. The approximate proportion of mature eggs developing to free-swimming larvæ (blastulæ and gastrulæ) is given.

TABLE XI.

N/260 BUTYRIC ACID.)

Time of Exposure.	Proportion of Mature Eggs Developing to Larvæ.				
	Series 1 (Aug. 31.)	Series 2 (Sep. 1).	Series 3 (Sep. 1).	Series 4 (Sep. 2.)	Series 5 (Sept. 2).
1 m.	0	0	1 or 2 larvæ	1 blastula	<1%
2 m.	2 or 3 larvæ	0	*ca.* 1%	*ca.* 1%	2–3%
3 m.	<1%	<1%	*ca.* 4–5%	1– 2%	20–30%
4 m.	<1%	*ca.* 1%	*ca.* 10%	5–10%	55–60%
5 m.	*ca.* 1%	*ca.* 10%	10–15%	20–30%	75–85%
6 m.	*ca.* 5%	*ca.* 50%	20–30%	30–40%	80–90%
7 m.	20–30%	70–80%	40–50%	20–25%	35–40%
8 m.	*ca.* 50%	65–75%	50–60%	15–20%	20–30%
10 m.	80–90%	65–75%	*ca.* 60%	*ca.* 1%	10–15%
12 m.	30–40%	25–35%	40–50%	1 blastula	0
15 m.	<1%	*ca.* 1%	20–30%	0	0

The close parallelism between these experiments and those of warming to 32° or 33° will at once be noted. With brief exposure there is the same simple membrane-formation followed by breakdown without development; as the exposure is prolonged there is a progressive increase in the proportion of favorably developing eggs up to an optimum; then follow a decrease and eventual failure to develop. More detailed observations show that the rate and regularity of cleavage show a corresponding steady improvement up to an optimum which is again followed by a decline.

The following observations show the condition of the eggs in the second series of September 1, at about four hours after the treatment with butyric acid (Table XII.).

The optimum time of exposure shows somewhat more variability in these series than is usually the case with exposure to

warm sea-water (32°); in all five, however, the optimum lay between five and ten minutes.[1] There is thus an approximate constancy in the time of exposure required to induce complete development with solutions of this concentration. Probably

TABLE XII.

N/260 BUTYRIC ACID.

Time of Exposure.	Condition of Eggs 4 Hours after Treatment, and Proportion forming Larvæ.
1 m.	All eggs have membranes; most are irregular or amœboid in form; none are cleaved. No larvæ.
2 m.	Similar to 1 m. lot, but a few eggs (*ca.* 2–3 per cent.) are in the 2-cell stage. No larvæ.
3 m.	Generally similar to the 2 m. lot, but the cleavages are more numerous (*ca.* 10–15 per cent.), mostly 2-cell with a few 4-cell stages. Very few larvæ (< 1 per cent.).
4 m.	Cleavages are more numerous and advanced; 40–50 per cent. are cleaved, mostly 2 and 4-cell, with a few 8-cell stages. Larvæ still few (*ca.* 1 per cent.).
5 m.	Cleavage is more advanced than in the 4 m. lot; *ca.* 50 per cent. are cleaved, largely 8- and 16-cell stages. *Ca.* 10 per cent. form larvæ.
6 m.	Most eggs are cleaved (*ca.* 70–80 per cent.), many in 16- to 32-cell stages. *Ca.* 50 per cent. form larvæ.
7 m.	Almost all eggs are cleaved (90 per cent. or more), many in normal-looking 16- to 32-cell stages. 70–80 per cent. form larvæ.
8 m.	Similar to 7 m. lot; most eggs are in 16- to 32-cell stages. 65–75 per cent. form larvæ.
10 m.	Cleavages are fewer and less advanced; *ca.* 70–75 per cent. are cleaved, mostly 4- and 8-cell stages. 65–75 per cent. form larvæ.
12 m.	Comparatively few cells are cleaved; *ca.* 10 per cent. are in 2- or 4-cell stages, largely irregular; the rest uncleaved. 25–35 per cent. form larvæ.
15 m.	Almost all eggs remain uncleaved, and many show the beginnings of surface-disintegration. Few form larvæ,—*ca.* 1 per cent.

Controls: Unfertilized eggs disintegrate without membrane-formation or development. Nearly all sperm-fertilized eggs develop to larvæ.

[1] This variability may be due partly to the fact that on account of the lateness of the season and consequent scarcity of ripe starfish the eggs used in these experiments came from fewer animals; thus in Series 1, 4, and 5, eggs from only one starfish were used in each case, and in Series 2 and 3 from three. In the earlier experiments with warm sea-water the mixed eggs from several animals were used in each series.

an inverse relation exists between the concentration of fatty acid and the time of exposure required to produce a given effect. Systematic experiments to determine the character of this relation have not yet been carried out, but there are some observations bearing on this question. In several of my experiments in the summer of 1912 starfish eggs exposed for only one minute to acetic or butyric acid of *ca.* $n/176$ concentration (6 c.c. $n/10$ acid *plus* 100 c.c. sea-water) formed a large proportion of larvæ.[1] Lyon observed some years ago that the exposure required to induce parthenogenesis in *Arbacia pustulata* by means of weak solutions of HCl in sea-water decreased with increase in the concentration of acid up to a certain point.[2] The minimum exposure to $n/260$ butyric acid required to form membranes is very brief in starfish eggs. Experiments last summer showed that while 10 seconds was insufficient to form membranes in more than a few eggs (*ca.* 10 per cent.), with 20 seconds all formed membranes, followed by the typical irregular changes of form and breakdown. After one minute's exposure to $n/260$ butyric acid an occasional egg may form a blastula; yet in the series showing the shortest optimum exposure of any performed last summer (No. 5, Sept. 2) at least 3 minutes was required to enable any considerable proportion of eggs to develop to a larval stage. The parallelism between the effects of high temperature and of weak fatty acid solutions indicates that the two agents act by producing the same kind of change in the egg-system. More detailed experiments to determine the influence of concentration as well as time on the action of this and other cytolytic substances remain to be carried out, and their results will probably throw further light on the nature of this change.

Effects of Membrane-formation by Heat or Fatty Acid Combined with After-treatment by the Same Agent.

The fact that a longer treatment with the membrane-forming agent produces the same effect as a short treatment combined with after-exposure to hypertonic sea-water or cyanide suggests that a suitable after-treatment with the membrane-forming

[1] Cf. *Journal of Experimental Zoölogy*, 1913, Vol. 15, pp. 41, 42.

[2] Lyon, *American Journal of Physiology*, 1903, Vol. 9, p. 310.

agent itself should have a corrective effect similar to that exerted by the agents just named. If the effect of the initial or membrane-forming treatment is to cause a partial activation which requires later to be completed by the after-treatment, we should expect it to be a matter of indifference (within certain limits of time) whether the activation is completed in one stage—*e. g.*, by a continuous warming to 32° for 8 minutes—or in several; development ought to follow equally well if the eggs are returned to sea-water after an exposure just sufficient for membrane-formation, and afterward again exposed to the same treatment for an appropriate length of time. Experiment shows that it is in fact possible to substitute for the after-treatment with hypertonic sea-water or cyanide a brief exposure either to warm sea-water or to $n/260$ butyric acid. We have here clear indication that the essential changes produced in the egg by after-treatment with an agent like hypertonic sea-water are not qualitatively different from those caused by the first or membrane-forming treatment, but serve simply to renew and bring to its completion a process which has been initiated by the first treatment but prematurely arrested by the early return to normal sea-water. According to this conception the whole activation-process is unitary in nature and does not consist of two qualitatively distinct and mutually complementary processes, as Loeb has maintained on the basis of his experiments with sea-urchin eggs.

The following series (Table XIII.) illustrates the effects of treating eggs, in which membranes have been formed by 3 minutes' exposure to 32°, a second time with sea-water at 32° for 4 minutes; the second exposure was made at varying intervals after the first, ranging from 9 minutes to nearly 4 hours.

Table XIII.

After-treatment with Sea-water at 32°.

June 24. Eggs from several starfish were exposed, about 35 minutes after removal from the animals, to sea-water at 32° for 3 minutes (11.12–11.15 A.M.), and then returned to sea-water. Part of these eggs were left permanently in sea-water for control; the rest were again exposed to 32° for 4 minutes, successive portions being thus treated at 10-minute intervals until well after the separation of the second polar body. The condition of the maturing eggs at the time of the second treatment is indicated in the first column.

Control lots of eggs were exposed (for purposes of comparison) to 32° for single continuous periods ranging from 2 minutes to 10 minutes.

After-exposures to 32° at Following Times after First Exposure.	Results (Condition of Eggs *ca.* 4 Hours Later, and Proportion Forming Larvæ.
1. Control: no second exposure	All mature eggs have membranes but are uncleaved and largely irregular in form. None form larvæ.
2. 9 m. (11.24–11.28) (no polar bodies at 11.28)	Marked contrast to control; almost all eggs are cleaved, largely to 32- or 64-cell stages. *Ca.* 70–80 per cent. form larvæ many of which swim at the surface of the water.
3. 19 m. (11.34–11.38) (first polar bodies beginning to separate at 11.38)	Cleavage is less advanced than in Experiment 2, and a minority are uncleaved. Somewhat fewer larvæ (*ca.* 65–75 per cent.).
4. 29 m. (11.44–11.48) (first polar bodies in all eggs at 11.48)	Cleavages are fewer and less advanced than in Experiment 3. Most eggs are uncleaved. Larvæ fewer (*ca.* 30–40 per cent.).
5. 39 m. (11.54–11.58) (all with first polar bodies, none with second at 11.58)	Contrast to Exp. 4; great majority are uncleaved and largely irregular; a few 2- and 4-cell stages present; few form larvæ (*ca.* 5 per cent.).
6. 49 m. (12.04–12.08) (*ca.* 50 per cent. have second polar body at 12.08)	Similar to Exp. 5 but with fewer cleavages. Larvæ also are fewer (*ca.* 2–3 per cent.).
7. 59 m. (12.14–12.18) (all eggs have second polar bodies)	Nearly all are uncleaved; largely irregular or fragmented. Almost no larvæ (only one feeble blastula seen).
8. 1 h. 9 m. (12.24–12.28)	All are uncleaved but irregular forms are fewer. No larvæ.
9. *ca.* 3 h. (2.59–3.03)	Similar to Exp. 8.

Controls with one exposure to 32°: eggs exposed 7 minutes continuously (11.12–11.19) gave *ca.* 90 per cent. larvæ. With 4 minutes' exposure few eggs (*ca.* 2–3 per cent.) formed larvæ. Controls of unfertilized and sperm-fertilized eggs were normal.

Experiments similar to the above were performed with preliminary exposures to 32° of 2, 3 and 4 minutes, followed by after-exposure to 32° as above for 4 minutes (in one series for five minutes), all of which gave the same general result. Apparently it is a matter of indifference whether the second exposure to 32° follows immediately after the first or at an interval, provided that the second exposure takes place before the separation of the first polar body. After this event there follows a decided and rapid decline in the favorability of the response to the after-warming treatment, and after the separation of the second polar body after-warming is apparently quite ineffective. As I described in my former paper on this subject, the susceptibility to parthenogenesis by temporary continuous warming always undergoes marked and rapid decrease at the time of the maturation-divisions.[1] The above decline in the response to after-warming is evidently the same phenomenon. A similar decrease in the susceptibility of the eggs to sperm-fertilization also takes place at about the same time, although this decrease is not so pronounced as in the case of parthenogenesis; thus it is usually possible to fertilize a certain variable proportion of starfish eggs (not all) after maturation has been complete for some hours.[2] The fact that the general responsiveness of the egg to any activating agent undergoes a sudden decline at the time of separation of the polar bodies suggests either that some material necessary to development is then lost, or that a refractory state conditional on some other kind of change (possibly a change in the plasma-membrane) then develops. As already pointed out, the fact that sperm-fertilization is possible (although less favorable) at a time when the egg fails to respond to the parthenogenetic treatment suggests that some definite material playing an important part in development is introduced into the egg by the sperm. This is also indicated by the general fact that sperm-fertilization induces a more favorable development than artificial activation. It may be that this material is the same as some substance lost from the egg at the time of the maturation-divisions. Further research has to decide between these possibilities.

[1] *Loc. cit.*, 1908, p. 400.

[2] *Loc. cit.*, p. 411.

In the above described experiments the total optimum period of exposure to 32° is about the same (*ca.* 7 to 8 minutes) whether the exposure is continuous or in two stages. No doubt it would be possible to increase the number of stages to three or more, especially if lower temperatures (31° or 30°) were used, but no experiments of this kind have so far been attempted. Apparently what is essential is that the critical process begun by the warming should continue, at the given temperature, for a certain definite length of time, sufficient presumably to allow some critical chemical interaction to proceed to its completion. It is interesting to note that a preliminary warming which is too brief in itself to cause membrane-formation may nevertheless have the effect of shortening the period of after-warming necessary to cause complete development. In one experiment the preliminary exposure to 32° was only 2 minutes, a time insufficient for membrane-formation in more than very few eggs (*ca.* 1 per cent.); these eggs, however, when again exposed to 32° for 4 minutes, gave a considerable proportion of larvæ (5 to 10 per cent.); while eggs exposed to 32° for 4 minutes without any previous treatment formed membranes, but none developed to larvæ. A continuous single exposure of 6 minutes gave 25 to 35 per cent. of larvæ; this exposure was well below the optimum of 8 to 10 minutes at which 80 to 90 per cent. formed larvæ. This effect of the four minutes' after-exposure on eggs which otherwise showed no external change indicates that membrane-formation is not in itself a critical event, but simply an expression of a partial initiation of the general developmental process: *i. e.*, a partial activation has been accomplished, enabling the egg to carry out a few of the early steps in development.

Since brief exposure to weak fatty acid solution has the same physiological effect on the egg as brief warming, it would appear that the essential change produced in the egg-protoplasm by either form of treatment is the same; if so, after-treatment with warm sea-water should have a similarly favorable effect on eggs in which membranes were formed by fatty acid. The following series of experiments shows that this is the case (Table XIV.). The eggs, after membrane-formation by butyric acid, were after-treated with warm sea-water (32°) for periods ranging from 2

to 12 minutes. For comparison part of the eggs were after-treated with hypertonic sea-water and cyanide.

TABLE XIV.

n/260 BUTYRIC ACID WITH AFTER-TREATMENT WITH SEA-WATER AT 32°.

August 24. Eggs from one starfish were used. These eggs were not very favorable and a rather small proportion underwent maturation. They were exposed, about 45 minutes after removal, to *n*/260 butyric acid solution for one minute and then returned to normal sea-water. Twelve to sixteen minutes later they were after-treated as follows, with the results indicated.

After-treatment.	Results (Condition of Eggs 4–5 hours later, and Proportion of Mature Eggs forming Larvæ).
1. None (control treated with butyric acid alone)	Typical fertilization-membranes in all mature eggs; later the eggs assume irregular forms and break down. None form larvæ.
2. Hypertonic sea-water (250 c.c. s. w. + 40 c.c. 2.5 m NaCl) for 30 min.	Markedly favorable effect: most mature eggs are cleaved to *ca.* 32-cell stage. 20–30 per cent. form larvæ.
3. M/1000 KCN in sea-water for 30 min.	Eggs cleave as in Experiment 2. *Ca.* 25–30 per cent. form larvæ.
4. Sea-water at 32° for 2 min.	After four hours most eggs are irregular and uncleaved; a few are cleaved. Very few form larvæ (< 1 per cent.).
5. 32° for 3 min.	Like Exp. 4, but more eggs are cleaved. Few larvæ, —1 per cent. or less.
6. 32° for 4 min.	Cleavages are more numerous than in Exps. 4 and 5. *Ca.* 5 per cent. of mature eggs form larvæ.
7. 32° for 5 min.	*Ca.* 20–30 per cent. are cleaved. *Ca* 5 per cent. form larvæ.
8. 32° for 6 min.	Cleavages are more numerous than in Exp. 7. *Ca.* 40–50 per cent. larvæ.
9. 32° for 7 min.	Most eggs in 16- to 64-cell stages. 50–60 per cent. form larvæ.
10. 32° for 8 min.	Like Exp. 9, but somewhat less favorable. *Ca.* 50 per cent. form larvæ.
11. 32° for 10 min.	After five hours few eggs are cleaved and cleavages are less advanced than in Exps. 9 and 10. *Ca.* 10–15 per cent. form larvæ.
12. 32° for 12 min.	Almost none have cleaved after five hours. Practically none form larvæ (one blastula seen).

For comparison eggs were exposed to 32° without previous membrane-formation for 4, 5, 6, 7, 8, and 10 minutes; the optimum exposure was 8 minutes at which 50–60 per cent. of the mature eggs formed larvæ. A sperm-fertilized control also yielded numerous larvæ.

After-exposure to 32° for the proper time thus greatly increases the proportion of favorably developing eggs. No marked improvement is seen until the duration of after-exposure reaches four minutes; with longer exposures the proportion of eggs forming larvæ shows progressive increase up to an optimum at about seven minutes; a decline then follows; an exposure of 10 minutes effects only slight improvement, and one of 12 minutes appears ineffective. Similar results, differing slightly in detail in different series, were obtained in eight other series of experiments. In general, after the preliminary membrane-formation by one minute's exposure to *n*/260 butyric acid, the time of exposure to 32° required for optimal development was found to range from 5 to 7 minutes; one minute's exposure to *n*/260 butyric acid appears thus physiologically equivalent to warming at 32° for the same or a somewhat longer period. After-treat-

Table XV.

n/260 Butyric Acid with After-treatment with Sea-water at 34°.

August 27. The eggs from one starfish were used. The eggs were few in number, but the majority showed normal behavior. They were exposed to *n*/260 butyric acid for one minute and then returned to sea-water. Later (within 20 minutes) portions were exposed to hypertonic sea-water, cyanide, and warm sea-water as indicated.

After-treatment.	Results.
1. None (control)	Typical membrane-formation, followed by breakdown of almost all eggs. One blastula found.
2. Hypertonic sea water for 30 m.	35–45 per cent. of the eggs form larvæ.
3. *n*/1000 KCN for 30m.	*Ca.* 50 per cent. of all eggs form larvæ.
4. 34° for 1 min.	Only a few eggs form larvæ: < 1 per cent.
5. 34° for 2 min.	Marked improvement: 20–30 per cent. form larvæ.
6. 34° for 3 min.	Larvæ are fewer than in Exp. 5: *ca.* 20 per cent.
7. 34° for 4 min.	Few eggs form larvæ: < 1 per cent.
8. 34° for 5 min.	Most eggs fail to divide; none form larvæ.

Warming at 34° without previous membrane-formation: Eggs were exposed to 34° in the usual manner for 2, 3, 4, 5, 6, and 7 minutes. The best development resulted from the 2- and 3-minute exposures, with respectively 25–35 per cent. and 35–40 per cent. of eggs forming larvæ; with the 5-minute exposure only 5 per cent. formed larvæ.

ment with sea-water at 31° and at 34° was also tried; the results were the same except that the after-exposure required at 34° was only a half to a third as long as at 32°, and at 31° about twice as long. The following series at 34°(Table XV.) is typical.

These results show that the effective duration of after-exposure at 34° is about one third of what it is at 32°; at 31° the best results were gained with after-exposures of 8 to 10 minutes. The temperature-coefficient of the physiological change resulting from the after-warming treatment is thus evidently of the same order as in the case of simple warming without previous membrane-formation. This of course is not surprising, since undoubtedly the same process is concerned in activation by heat whether this is preceded by another treatment or not.

It is thus plainly a matter of indifference, as regards the effect

Table XVI.

Both Membrane-formation and After-treatment by *n*/260 Butyric Acid

September 6. The eggs from one starfish were used; these were few in number, but almost all (*ca.* 90 per cent.) showed normal maturation, and in the sperm-fertilized control almost all formed larvæ. The eggs were exposed for one minute to *n*/260 butyric acid and returned to sea-water; part were left in sea-water as control; the remainder were again placed, 18 minutes later, in *n*/260 butyric acid, from which portions were returned to normal sea-water at the intervals indicated. These eggs developed as follows:

After-treatment.	Results (Condition of Eggs after 4 Hours and Proportion forming Larvæ).
1. None (control)	All show typical membrane-formation followed by irregular change of form and breakdown in nearly all eggs. Only one larva seen.
2. *N*/260 Butyric acid: 2 m.	A few eggs are cleaved. *Ca.* 10–15 per cent. form larvæ.
3. Butyric acid: 4 m.	Cleavages more numerous and more regular than in Exp. 2. *Ca.* 40–50 per cent. of eggs form larvæ.
4. Butyric acid: 6 m.	Cleavages still more numerous: Most eggs form larvæ (70–80 per cent.).
5. Butyric acid: 8 m.	Like Exp. 4, but fewer eggs form larvæ (50–60 per cent.).
6. Butyric acid: 10 m.	Cleavages are fewer and slower. 25–35 per cent. of eggs form larvæ.
7. Butyric acid: 12 m.	Cleavages are still fewer. *Ca.* 10 per cent. of eggs form larvæ.
8. Butyric acid: 15 m.	Practically none are cleaved. No larvæ.

produced by this form of after-treatment, whether the membrane-formation is induced by heat or by fatty acid; in either case warming for a few minutes completes the process of activation and enables the eggs to develop favorably. Precisely the same effect is gained by after-exposing eggs, in which membranes have been formed by either method, to weak solutions of fatty acid for a brief period; the effects of such treatment are in all respects similar to those of after-warming. This is illustrated by the following experiment (Table XVI.).

It is clear that in the time-relations of its action as well as in its other characteristics, this form of after-treatment resembles closely that with warm sea-water. It is also possible to treat the eggs first with warm sea-water and then after-treat with butyric acid solution; precisely the same results follow as in the experiment just described. This is illustrated by the following series (Table XVII.).

Table XVII.

Brief Exposure to 32° with After-treatment by *n*/260 Butyric Acid.

September 7. The eggs from one starfish were used; eggs were few but apparently normal, over 90 per cent. showing normal maturation, and sperm-fertilization resulting in a large proportion of larvæ. The eggs were exposed to sea-water at 32° for 3 minutes, then returned to sea-water at normal temperature, and 16 minutes later placed in *n*/260 butyric acid solution, from which they were again returned to sea-water after the times indicated.

After-treatment.	Results.
1. None (32° for 3 m. alone)	No development; only a small proportion form membranes.
2. *N*/260 butyric acid: 2m.	All form membranes but few are cleaved after three hours. *Ca.* 5 per cent. form larvæ.
3. Butyric acid: 4 m.	A large proportion (50–60 per cent.) are cleaved after three hours. More than 50 per cent. form larvæ.
4. Butyric acid: 6 m.	Most eggs are cleaved after three hours. 70–80 per cent. form larvæ.
5. Butyric acid: 8 m.	In contrast to Exp. 4, few eggs are cleaved after three hours, and only 1–2 per cent. form larvæ.
6. Butyric acid: 10 m.	No eggs cleave within three hours. None form larvæ.

The favorable effect of this after-treatment is evident. It will be noted that the three minutes' exposure to 32° was insufficient for membrane-formation in most eggs; but the effect

of this preliminary treatment is seen in the fact that an after-exposure of only 4 minutes was sufficient to induce development to larval stages in more than half of the eggs. After-exposure to butyric acid solution has the same favorable effect when the preliminary warming is sufficient to form membranes in all eggs; in a second similar series on September 12 the eggs were exposed for 4 minutes to 32° and all mature eggs thus treated formed membranes; without any after-treatment almost none (less than 1 per cent. formed larvæ, but with an after-treatment of 4 to 8 minutes with *n*/260 butyric acid favorable development took place in a large proportion of eggs.

General Discussion and Conclusion.

The interchangeability of the treatments with warm sea-water and butyric acid solution indicates that both agents produce their effect by inducing the same kind of change in the egg-system. This change is evidently of a "releasing" kind, and initiates the sequence of developmental processes; these, once started, continue automatically to their conclusion. Probably their most distinctive peculiarity is the highly specific character of the chemical transformations that take place. From the food contained as reserves in the egg, or taken in from the surroundings, the developing germ builds up the specific compounds which form the structural basis of the organism; this synthetic process, in the case of the chief structure-making compounds, the proteins, undoubtedly starts—as in the constructive metabolism of the adult animal—with the amino-acids, which are recombined in the specific manner predetermined by the chemical organization of the germ. Bodies of the most highly specific and individualized physical and chemical properties are thus built up and laid down in definite positions as development proceeds. Their properties and their spacial disposition determine at any time the character of the transformation undergone by the building material which is being incorporated. According to this conception it is the chemical specificity of these substances that determines the specific character of development in the more evident or morphological sense,[1] *i. e.*, why the egg gives

[1] Reichert's work on the crystal-forms of haemoglobin and other complex compounds from different species of animals and plants constitutes perhaps the

rise to an individual of the same species; and we must therefore be prepared to find among the earliest chemical changes associated with development, interactions of a specific kind—*i. e.*, specific in the sense in which the interaction of antigen and antibody is specific—between complex substances already present in the egg. There is now definite experimental evidence that such reactions do in fact constitute an essential part of the fertilization-process.[1] Specific substances which apparently unite in fertilization (since after fertilization they are no longer demonstrable) are present in the unfertilized mature egg; one of these ("fertilizin") may be largely removed from the egg by washing, and when this is done fertilization is prevented. If such specific unions are essential to fertilization, we must conclude that the specific substances concerned in this process are in some way kept from interaction in the resting mature egg, and that the activating agent removes this hindrance to interaction. The question which I wish briefly to discuss in this section relates to the nature of this inhibiting condition, and the manner in which the activating agent effects its removal.

The nature of the effects following exposure of unfertilized eggs to temperatures of 30°–35° indicates clearly that activation does not depend on simple acceleration of some chemical process, *e. g.*, oxidation, which is already proceeding in the egg, since in this case the temperature-coefficient of the activation-process would presumably show the usual value of $Q_{10} = 2–3$. It is also evident that heat-coagulation is not concerned, since these temperatures are too low, and the readiness with which the activation process can be arrested by cooling and renewed by a second warming shows that its basis is some effect which is completely reversible by change of temperature. These characteristics, high temperature-coefficient and reversibility with change of temperature, are however shared by the typical melting and gelation (sol-gel transformation) exhibited by solutions of gela-

best evidence of this. The morphological characters of crystals and crystal-aggregates varies with their chemically specific ("species-specific") character in a definite and constant manner. It is fair to assume that the influence of these compounds in determining organic structure depends largely on the kind of aggregates they form. *Cf.* Reichert: *Science*, 1914, N. S., Vol. 40, page 649.

[1] *Cf.* F. R. Lillie, *Journal of Experimental Zoology*, 1914, Vol. 16, p. 523.

tine, agar, soaps, lipoids and other hydrophilous colloids. The relations of temperature to this process show in fact a close resemblance to those described above for the activation-process. One striking peculiarity of melting and gelation is that both processes take place gradually; when (*e. g.*) a gelatine sol is brought below the gelation-temperature and the conditions are then kept constant, the actual solidification takes place only after the lapse of a considerable period of time. The time required to reach the gelation-stage decreases rapidly as temperature is lowered; thus Levites found that a gelatine sol kept undisturbed at 26° took 26 hours to gelatinize, at 25° only 11 hours.[1] The first observable change in the solution is an increase in viscosity; this continues until the system sets; the setting represents the end-stage of the whole process, whose course can thus be traced by successive viscosity-determinations. Gelation is thus equivalent to a progressive increase in viscosity to a final stage at which the ordinary fluid mobility is lost.[2] It is found that above a certain temperature the viscosity of the hydrosol undergoes no change with time; but if the temperature is lowered a critical point is eventually reached below which the viscosity undergoes steady increase (at a rate dependent on temperature, presence of salts, reaction) until gelation occurs. The rate of this increase in viscosity (*i. e.*, of the gelation-process), $\Delta\eta/\Delta t$, shows a high temperature-coefficient. With a 1 per cent. gelatine solution Schroeder[3] obtained the following values for the viscosity at 21°, 24.8°, and 31° at different intervals after bringing the warm gelatine solution to the temperature of observation:

Interval.	Viscosity Observed at		
	21°.	24.8°.	31°.
5 min.	1.83	1.65	1.41
10 min.	2.10	1.69	1.41
15 min.	2.45	1.74	1.42
30 min.	4.13	1.8	1.42
60 min.	13.76	1.9	1.42

Thus while at 31° the viscosity undergoes no change with time,

[1] Levites, *Kolloid-Zeitschrift*, 1907, Vol. 2, p. 211.

[2] *Cf.* Schroeder, *Zeitschrift für physikalische Chemie*, 1903, Vol. 45, p. 75; Levites: *loc. cit.*, p. 209; Freundlich, "Kapillarchemie," 1909, pp. 416 ff.

[3] Schroeder, *loc. cit.*, p. 88.

at the lower temperatures there is a steady increase. If we take comparatively short time intervals, *e. g.*, 10 minutes, we find that the value of $\Delta\eta/\Delta t$ at 21° $\left(\frac{2.45 - 1.83}{10} = 0.062\right)$ is about seven times greater than at 24.8° $\left(\frac{1.74 - 1.65}{10} = 0.009\right)$. In other words, a difference of about 4° increases the average rate of the gelation-process from six to seven times. What is true of the gelation-process is also true of the inverse degelation or melting process, whose rate increases at a similarly rapid rate with rise of temperature above the critical maximum at which the system remains permanently in the gel state.[1]

In starfish eggs the rate of the activation-process, at temperatures between 30° and 36°, shows a similar proportionate increase with a given rise of temperature, as will be seen by reference to Table X.; *i. e.*, the temperature-coefficients of the two processes, gel-sol transformation, and activation of the egg under the influence of high temperatures, are similar in their order of dimensions; thus a rise of 4° shortens the time of exposure necessary to cause membrane-formation or development by six to ten times. On the assumption that some specific chemical interaction is the essential change in the initiation of development, such a result indicates that the rate of this interaction is dependent, in the case of parthenogenesis by warming, on the rate of some process involving either degelation or decrease in the viscosity of some portion of the colloidal system of the egg. This is as much as can be inferred on the basis of these facts alone. If we also take into account the other methods by which membrane-formation and activation can be induced, we are led to the further inference that this colloidal change affects chiefly if not exclusively the surface-layer (cortical zone or plasma-membrane) of the egg. Thus typical membrane-formation can be induced by brief treatment with pure isotonic solutions of neutral salts.[2]

[1] On account of the hysteresis of the gelatine system, the melting temperature is typically several degrees higher than the solidification-temperature; it is also higher after the gel has stood some time than immediately after solidification. *Cf.* Pauli (Pascheles): *Archiv f. d. ges. Physiologie*, 1898, Vol. 71, p. 336.

[2] R. S. Lillie: *American Journal of Physiology*, 1910, Vol. 26, p. 106. The fact

whose action is certainly superficial, as well as by substances like fatty acids, weak bases, and lipoid-solvents, which readily penetrate the plasma-membrane. Those neutral salts of sodium and potassium which are the most effective in inducing membrane-formation, iodides and thiocyanates, are also the most effective in lowering the melting points of protein gels and in promoting water-absorption by such gels.[1] Such facts suggest that the salts act in a way similar to that of high temperatures, *i. e.*, by furthering degelation of surface-structures or absorption of water in the surface-layer of the egg. The effect of such an increase in water-content would be to increase the general permeability of this region, since according to the experiments of Bechhold, Ruhland, and others[2] the permeability of gels to diffusing substances, especially to colloids, is a direct function of their water-content.

High temperature, according to this interpretation, acts like other parthenogenetic agents, by increasing the permeability of the surface-layer,—this effect resulting directly from some change in the nature of a degelation or decrease in the viscosity of the colloidal system in this region. Apparently the immediate effect of this change is to allow a chemical interaction to take place between substances which in the normal resting state of the surface-layer are kept apart. The general fact that identical physiological effects may be produced by lipoid-solvents, and by substances which appear to alter the membrane by interacting chemically with its constituents,[3] indicates that the integrity of the plasma-membrane as a semi-permeable partition is the essential factor in preserving the resting condition of the egg.[4]

that this action can be prevented by anesthetics confirms the view that it depends on an increase in the permeability of the plasma-membrane: *cf.* my recent paper in the *Journal of Experimental Zoology*, 1914, Vol. 16, p. 591.

[1] *Cf.* Pauli (Pascheles): *Archiv f. d. ges. Physiologie*, 1898, Vol. 71, p. 333; Levites: *loc. cit.*; Pauli and Rona, *Beiträge zur chemischen Physiologie u. Pathologie*, 1902, Vol. 2, p. 4.

[2] Bechhold u. Ziegler, *Zeitschr. f. physik. Chem.*, 1906, Vol. 56, p. 105; also, "die Kolloide in Biologie u. Medizin," 1912, p. 48. Ruhland: *Biochemische Zeitschrift*, 1913, Vol. 54, p. 59; Freundlich, *Kapillarchemie*, pp. 515 *seq.*

[3] When membrane-forming substances act by combining chemically with egg-constituents, it is to be expected that the rate of action will vary with temperature in accordance with the chemical temperature-coefficient. *Cf.* the experiments of Loeb and Hagedoorn, "Artificial Parthenogenesis and Fertilization," page 146.

[4] *Cf.* my paper, *Amer. Journ. Physiol.*, 1911, Vol. 27, p. 289.

Hence it is a matter of secondary importance in what manner this semi-permeability is temporarily destroyed, provided that the condition of increased permeability lasts long enough—not too long—and is not associated with irreversible changes making recovery impossible. It is presumably during this stage of increased permeability that the above specific interaction takes place; this process requires time, and its rate will be a function of the rate at which the two interacting substances can come together; this second rate will be a function of the viscosity or gelation-state of the protoplasmic system at the site of interaction,—hence its dependence on temperature, as seen above. When this critical interaction has taken place, there follows at once the characteristic change of physiological activity normally resulting from fertilization; membrane-formation and the other events preparatory to cell-division occur and the developmental process proper is initiated. How far development proceeds, however, depends on the degree of completion of the primary specific reaction; hence for complete activation the exposure to the membrane-forming condition must have a certain minimal duration, and in case the preliminary exposure is insufficient some after-treatment may be necessary to complete the process. This after-treatment may be of the same kind as the preliminary membrane-forming treatment, or it may be of entirely different kind—*e. g.*, hypertonic sea-water, cyanide, an anaesthetic, etc. But there seems to be no need of assuming that its direct physiological effect is qualitatively different from that of the membrane-forming agent.[1] It merely renews and brings to completion a process already initiated by the first treatment.

Comparative study of the conditions of both normal and

[1] The above experiments are a sufficient justification of this contention. But they do not explain why, for instance, after-treatment with cyanide, which by itself does not induce membrane-formation in starfish eggs (*cf. Journal of Experimental Zoology*, 1913, Vol. 15, p. 38), is so effective. Clearly the condition of the egg after membrane-formation is altered so that the activation-process may then be influenced by agents which previously had no effect upon it (as cyanide, alcohols, or hypertonic sea-water in brief exposure). Sensitization to these agents seems to be involved in the process of membrane-formation, but the basis of this effect can not be defined at present. There is, however, no necessary inconsistency between these facts and the conception that the activation-process is essentially unitary in character in the above sense. The case of hypertonic sea-water offers certain special problems, which are partly discussed below.

artificial activation ought to yield data from which by elimination the essential factors common to the two processes may be determined. Judging from the data available at present, the most general common feature appears to be the initial increase in permeability.[1] It is not yet clear, however, how this change can be the means of initiating the specific interaction assumed. The substances which interact are assumed to be present in advance in the egg; how is their interaction prevented by the existence of a semipermeable surface layer? The connection between change of permeability and activation is probably indirect; and the analogy to stimulus and response in the general stimulation-process of irritable tissues still seems the best adapted to throw light on this question.[2] In stimulation an electrical depolarization of the plasma-membranes of the irritable elements is apparently the critical event; in some way this change enables the characteristic response of the irritable system to take place. Similarly in the initiation of development in the unfertilized egg. The agents which induce membrane-formation in eggs have typically a depolarizing action on irritable cells like muscle-cells—*i. e.*, cause a negative electrical variation.[3] Such a change appears to result whenever surface-permeability is increased; and it seems therefore probable that this depolarization, as such, is what enables the union of specific substances—the first step in activation—to take place. We may assume that one of the interacting substances is situated immediately beneath the electrically polarized surface-film of the egg, that it is a negative colloid, and that its tendency to unite with some amboceptor-like body also present in this region is compensated by the electrostatic attraction between it and the layer of

[1] *Cf.* my paper just referred to. In a recent paper Gray confirms McClendon in finding a temporary increase in the electrical conductivity of sea-urchin eggs immediately after sperm-fertilization. *Cf.* Gray, *Journ. Mar. Biol. Ass.*, 1913, Vol. 10, p. 50; McClendon, *American Journ. Physiol.*, 1910, Vol. 27, p. 240.

[2] I have discussed this analogy in more detail in the paper above cited (footnote 2, p. 296); also in the *Journal of Experimental Zoology*, 1913, Vol. 15, p. 23.

[3] For the action of cytolytic substances in producing local negative variation, *cf.* Straub, *Archiv f. exp. Path. u. Pharm.*, 1902, Vol. 48, p. 1; *Zeitschr. f. Biol.*, 1912, Vol. 58, p. 251; Henze: *Arch. f. d. ges. Physiol.*, 1902, Vol. 92, p. 451; Hermanns: *Zeitschr. f. Biologie*, 1912, Vol. 58, p. 261; Allcock; *Proc. Roy. Soc.*, B, 1906, Vol. 77, p. 267; *Journal of Physiology*, 1906, Vol. 33, p. xxviii; Evans, *Zeitschr. f. Biol.*, 1913, Vol. 59, p. 397.

positive ions immediately external to the egg-surface. Depolarization would then permit interaction to take place.[1] Such a conception, while in a sense diagrammatic, helps at least to explain how a non-specific agency, provided it only alters sufficiently the boundary-layer of the egg, can be the means of initiating such a highly specific process as development.

The discussion of this question can hardly be considered complete without some reference to the case of hypertonic sea-water. As Loeb has shown, exposure to this agent forms a supplementary treatment which is remarkably favorable with some eggs, especially sea-urchin eggs. This treatment seems to occupy a special position among the parthenogenetic agents. It may either precede or follow the membrane-forming treatment,[2] and in some way it puts the egg into a condition which is favorable to subsequent development; this action seems quite independent of the nature of the membrane-forming or activating agent, and so far it has received no satisfactory explanation. Loeb has shown that a purely physical abstraction of water is not the only factor concerned; a chemical factor, apparently involving oxidation, is essential; free oxygen must be present during the treatment, and the effective times of exposure vary at different temperatures according to the chemical temperature-coefficient.[3] Some hypothesis as to its mode of action seems required; and I suggest the following, which is consistent with the foregoing point of view, and has not, to my knowledge, yet been put forward.

[1] The inorganic analogy would be, *e. g.*, the interaction between solution and metal at the surface of the plate in a battery when the circuit is closed. While the battery is at rest (with open circuit), interaction between (*e. g.*) sulphate ions and zinc is prevented by the polarization at the surface of the zinc plate. The tendency to this ionic interaction is compensated by the polarization, the zinc ions being held back by the negatively charged plate. Similarly, *mutatis mutandis*, with the reactions at the cell-surface, or other surfaces (adsorption-surfaces) within the cell. The facts of stimulation afford in general strong evidence that the chemical processes in the living cell are largely dependent on changes in the electrical polarization of the limiting membranes. *Cf.* my paper in the *Journal of Biological Chemistry*, 1913, Vol. 15, p. 237. Also, for a more general discussion of this question, the article entitled "The Physico-chemical Conditions of Stimulation," in the *Popular Science Monthly*, 1914, p. 579.

[2] *Cf.* Loeb, "Artificial Parthenogenesis and Fertilization," Chapter 11; *Archiv für Entwicklungsmechanik*, 1914, Vol. 38, p. 409.

[3] "Artificial Parthenogenesis and Fertilization," Chapter 11.

It is to be assumed that the activation-process—as the earliest step in development, an essentially constructive process—involves syntheses of some kind. Now the intracellular as well as other organic syntheses consist as a rule, in the union of two or more molecules, with loss of water, to form larger molecules,—as in the formation of fats from glycerol and acids, of starch and glycogen from sugar, of polypeptides and proteins from amino-acids, etc. In order to account for the readiness with which these condensations occur in cells, it seems necessary to assume that the protoplasm is the seat of energetic dehydrations, probably in certain localized situations (possibly at membranes or other adsorption-surfaces). The artificial enzymatic synthesis of triolein from glycerol and oleic acid has been found to take place readily only when water is removed as completely as possible from the reacting mixture.[1] Hence the synthesis of fats by enzyme action in cells is intelligible only on the assumption that in the region of their formation there is energetic abstraction of water or dehydrolysis. Certain biological facts indicate that partial removal of water from cells is favorable to syntheses of the above kind. According to Overton, plasmolysis of plant-cells furthers the formation of starch in chloroplasts.[2] Butkewitsch also finds that the formation of starch in the amylase-rich cortex of certain plants (*Sophora*, *Robinia*) is promoted by placing in strong sugar-solutions (10–20 per cent. dextrose and saccharose).[3] The observations of Pavy and Bywaters and of Rubner on the formation of glycogen by yeast cells in strong sugar solutions constitute probably a further instance of the same phenomenon.[4] In general loss of water will

[1] *Cf.* the papers of Pottevin: *Comptes rendus de l'Acadêmie*, 1903, Vol. 136, p. 1152, and 1904, Vol. 138, p. 378; Taylor, *Journal of Biological Chemistry*, 1906, Vol. 2, p. 87; Hamsik, *Zeitschr. f. physiol. Chemie*, 1909, Vol. 59, p. 1; Armstrong and Gosney, *Proceedings Roy. Soc.*, Ser. B, 1914, Vol. 88, p. 176.

[2] Overton, *Vierteljahrsschrift d. naturf. Ges. in Zürich*, 1899, Vol. 44, pp. 131–2.

[3] Butkewitsch, *Biochem. Zeitschr.*, 1908, Vol. 10, p. 314; *cf.* pp. 336 seq.

[4] Pavy and Bywaters, *Journal of Physiology*, 1907, Vol. 36, p. 149; Rubner, *Archiv für Physiologie*, Suppl. 1912, p. 252, and *ibid.*, Vol. for 1913, p. 244.

Pavy and Bywaters found that in pure dextrose solutions the deposition of glycogen in yeast cells increased rapidly with increase in the concentration of dextrose up to an optimum. In 2 per cent. solutions there was little effect; in 4 per cent., 8 per cent., and 16 per cent. solutions there was a rapid progressive increase in the quantity of glycogen laid down in the cells to a maximum of over 13 per cent.

be favorable to—since it will supplement—the action of any dehydrating mechanism; and it is possible that in the sea-urchin egg after membrane-formation the intracellular dehydration-processes are by themselves not quite energetic enough to effect the syntheses necessary for initiating development, but become so when supplemented by the action of the hypertonic sea-water; *i. e.*, this agent has the effect of reducing the concentration of water at the *locus* of the reactions sufficiently to enable syntheses to take place which otherwise are impossible under the conditions. It is significant that cell-division is started in the sea-urchin egg by simple membrane-formation, but fails to continue,—just as if there were some failure in the supply of the necessary constructive materials; partial abstraction of water rectifies this condition. Since oxygen is necessary to this corrective process, we may assume that the syntheses belong in part to the class designated by Schmiedeberg[1] as oxidative syntheses.

From this general point of view the action of hypertonic sea-water becomes in a measure theoretically intelligible and ceases to be merely a detached empirical fact. Certain avenues of experimental approach to the problem are also suggested.

Summary.

§ 1. The effects following exposure of maturing unfertilized starfish eggs to high temperatures (29–36°) vary in a constant manner with the times of exposure as follows. Below a certain minimal duration of exposure to any given temperature (*e. g.*, 32°), no visible change is produced in the egg; slightly longer exposures induce the formation of typical fertilization-mem-

(as compared with about 5 per cent. under normal conditions); in more concentrated solutions there was a decline. They also found that too long exposure to a favorable solution (10 per cent.) was unfavorable; thus yeast incubated in 10 per cent. dextrose for 2½ hours showed an increase in glycogen-content from 4.84 per cent. to 11.66 per cent.; four hours later there was a decline to 9.33 per cent. These facts show a suggestive parallel with the effects of hypertonic sea-water on sea-urchin eggs; here also there is no effect until a certain minimal osmotic pressure is reached; with further increase in osmotic pressure there is a rapid increase in favorability up to an optimum; still further increase is unfavorable. Also for a favorable concentration there is at any temperature a definite optimum time of exposure.

[1] *Cf.* Schmiedeberg, *Archiv f. exper. Pathologie u. Pharmakologie*, 1893, Vol. 31, p. 281.

branes, but the eggs fail to cleave and soon break down without development; in order to induce favorable development an exposure of three to four times the minimum for membrane-formation is required (*e. g.*, 7–8 minutes at 32°); more prolonged exposures are again followed by failure to develop.

2. Between 29° and 38° the times of exposure required to produce these effects decrease very rapidly with rise of temperature; on the average a rise of 1° approximately halves the exposure required for a given physiological effect (such as membrane-formation, or complete activation, or heat-inactivation). The activation-process thus exhibits a characteristically high temperature-coefficient ($Q_{10} = 200$–400).

3. The effects of exposure to weak butyric acid solution (*n*/260) vary with time of exposure in a similar manner,—brief exposure causing membrane-formation followed by breakdown, longer exposures causing cleavage and development to larval stages, and still longer exposures causing cytolysis without development.

4. The inference is that the same process is initiated in the egg by exposure to warm sea-water as by fatty acid solution. This process must proceed to a certain stage in order that activation may be complete; if arrested too soon (brief exposure) only partial activation (membrane-formation followed by breakdown) results.

5. Eggs in which membranes are formed by minimal exposure to warm sea-water or *n*/260 butyric acid, followed by return to sea-water, may be made to develop favorably by a second treatment with either warm sea-water or fatty acid solution, as well as by after-treatment with cyanide-containing or hypertonic sea-water. A favorable after-treatment may thus be of the same kind as the membrane-forming treatment.

6. The temperature-coefficient of activation by high temperatures is of the same order as that of the melting of gels or the decrease in the viscosity of gelatine solutions. The above high temperatures thus probably act by producing degelation-effects in the surface layer of the egg; increase of permeability, with consequent depolarization, is the result of this change.

7. A new hypothesis of the mode of action of hypertonic sea-water is put forward.